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Safety and immunogenicity of three different formulations of an adjuvanted varicella-zoster virus subunit candidate vaccine in older adults: A phase II, randomized, controlled study☆,☆☆

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ABSTRACT

Background: This study investigated the safety and immunogenicity of different formulations and schedules of a candidate subunit herpes zoster vaccine containing varicella-zoster virus glycoprotein E (gE) with or without the adjuvant system $AS01_B$.
Methods: In this phase II, single-blind, randomized, controlled study, adults aged ≥60 years ($N = 714$) received one dose of 100 μg gE/$AS01_B$, two doses, two months apart, of 25, 50, or 100 μg gE/$AS01_B$, or two doses of unadjuvanted 100 μg gE/saline. Frequencies of $CD4^+$ T cells expressing ≥2 activation markers following induction with gE were measured by intracellular cytokine staining and serum anti-gE antibody concentrations by ELISA.
Results: Frequencies of gE-specific $CD4^+$ T cells were >3-fold higher after two doses of all gE/$AS01_B$ formulations than after one dose of 100 μg gE/$AS01_B$ or two doses of 100 μg gE/saline. Frequencies were comparable after two doses of 25, 50, or 100 μg gE/$AS01_B$. Serum anti-gE antibody concentrations were comparable after two doses of 50 or 100 μg gE/$AS01_B$ and higher than in the other groups. Immune responses persisted for at least 36 months. Reactogenicities of all gE/$AS01_B$ formulations were similar but greater than with gE/saline.
Conclusions: The three formulations of gE/$AS01_B$ were immunogenic and well tolerated in adults aged ≥60 years. Two vaccinations with gE/$AS01_B$ induced higher immune responses than one and the dose of gE impacted humoral but not cellular immune responses (NCT00434577).

1. Introduction

Herpes zoster (HZ) is caused by reactivation of latent varicella-zoster virus (VZV) in the dorsal or cranial root ganglia, often many years after a primary VZV infection [1]. HZ is characterized by unilateral radicular pain and vesicular rash, and the most frequent complication is post-herpetic neuralgia (PHN), a chronic pain syndrome that can persist for years [1]. In healthy individuals, the estimated lifetime risk of developing HZ is 30% [1,2]. HZ incidence increases with age and is most common in adults aged ≥50 years [1–4]. HZ is also common in immunocompromised individuals [1].

☆ *Meetings*: This study was presented in part at the 36th International Herpesvirus Workshop, Gdansk, Poland in July 2011.
☆☆ *Trademarks*: Zostavax is a registered trademark of Merck & Co., Inc. Varilrix is a registered trademark of the GlaxoSmithKline group of companies.
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Cell-mediated immunity (CMI) plays a critical role in protection against HZ, whereas the role of humoral immunity is less clear [5,6]. With aging, CMI responses to VZV decline and HZ incidence increases, suggesting that HZ develops in older adults because their VZV-specific CMI falls below a critical threshold.

The Shingles Prevention Study, a large, randomized, double-blind, placebo-controlled study, showed that vaccination with a high dose of live-attenuated VZV vaccine (*Zostavax*™, Merck & Co., Inc.) reduces HZ incidence by 51% in adults aged ≥60 years and by 38% in adults aged ≥70 years [7]. The vaccine efficacy against HZ was 70% in persons aged 50–59 years [8]. In post-licensure studies, vaccination reduced the risk of HZ by 51% in adults aged ≥60 years [9], 48% in immunocompetent adults aged ≥65 years, and 37% in immunocompromised adults aged ≥65 years [10]. Thus, *Zostavax*™ has a modest efficacy against HZ that diminishes with age.

Recombinant subunit vaccines are an alternative approach for HZ prevention, especially for older adults for whom efficacy of live-attenuated vaccines is limited and for immunocompromised persons for whom live-attenuated vaccines are contraindicated [2]. One possible antigen is glycoprotein E (gE) because it is the most abundant glycoprotein on the surface of VZV virions and VZV-infected cells [11–13]. It is essential for viral replication and cell-to-cell transmission, which contributes to viral spread and pathogenesis of skin infection [11–13]. Furthermore, gE is a major target of anti-VZV cell-mediated and humoral immune responses [14]. However, an adjuvant may be necessary to elicit sufficient immune responses in humans. In mice, combining gE with an adjuvant enhanced gE cellular immunogenicity [15]. Recently, a phase I/II, open-label, randomized study showed that a subunit HZ vaccine candidate consisting of gE and the adjuvant system $AS01_B$ (gE/$AS01_B$) induced strong and durable immune responses in young and older adults and had an acceptable safety profile [16].

The aim of the current study is to evaluate the safety and immunogenicity of different schedules and formulations of gE/$AS01_B$ in adults ≥60 years of age.

2. Materials and methods

2.1. Study design and subjects

This was a phase II, single-blind, randomized, controlled study conducted at 11 centers in the Czech Republic, Germany, The Netherlands, and Sweden between February 14, 2007 and February 3, 2011 (ClinicalTrials.gov, NCT00434577). The study protocol was approved by the national independent ethics committees of the participating countries and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all subjects before study entry.

Healthy subjects aged ≥60 years at the time of the first study vaccination were eligible for inclusion in the study. Subjects were excluded if they had a history of HZ; were previously vaccinated against HZ or with any vaccine containing 3-O-desacyl-4′-monophosphoryl lipid A (MPL) or *Quillaja saponaria* Molina, fraction 21 (QS21, Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, MA); were allergic to any of the vaccine components; had received a vaccine (except influenza) within 2 weeks, an investigational or non-registered product, chronic immunosuppressants, or corticosteroids within 30 days, or immunoglobulins or a blood product within 3 months before the first study vaccine dose; or had a history of drug or alcohol abuse. Subjects were stratified by age (60–69 years and ≥70 years in a 1:4 ratio) and randomized (1:3:3:3:3) to receive two doses two months apart of unadjuvanted gE (100 μg gE/saline), two doses two months apart of adjuvanted gE/$AS01_B$ (25, 50, or 100 μg gE), or one dose of saline followed by one dose of 100 μg gE/$AS01_B$ two months later.

Blood samples were taken for immunogenicity analyses at months 0, 2, 3, 12, 24, and 36. A subset of subjects had additional blood samples taken one week after each vaccination for biochemical analyses.

2.2. Study vaccines and administration

All study vaccines were developed and manufactured by GlaxoSmithKline (GSK; Rixensart, Belgium). $AS01_B$ is a liposome-based adjuvant system containing the immunoenhancers MPL and QS21. The gE lyophilized pellet was reconstituted before injection with liquid $AS01_B$ or saline. All vaccines were administered intramuscularly (0.5 mL) in the deltoid region of the left arm. All subjects received one dose of vaccine or saline at month 0 and a second at month 2.

2.3. Safety and reactogenicity

Solicited local reactions (pain, redness, and swelling at the injection site) and general reactions (fatigue, fever, headache, and myalgia) were recorded by subjects on diary cards for seven days after each vaccination. Unsolicited adverse events (AEs) were recorded for 30 days after each vaccination and serious adverse events (SAEs) were recorded over the entire study period. Intensity of the solicited reactions was scored on a scale from 0 (absent) to 3 (severe). All solicited local reactions were considered vaccination-related, and causality of the solicited general reactions, unsolicited AEs, and SAEs was assessed by the investigators.

2.4. Assessment of cellular immune responses

The frequencies of gE-specific $CD4^+$ and $CD8^+$ T cells expressing at least two activation markers (among interferon-γ, interleukin-2, tumor necrosis factor-α, and CD40 ligand) per 10^6 cells were measured by intracellular cytokine staining as previously described [16–18]. Briefly, peripheral blood mononuclear cells were stimulated in vitro with a pool of 64 20-mer peptides that overlap by 10 amino acids (1.25 μg/mL each) and cover the entire gE ectodomain (Eurogentec) or with a lysate of VZV Oka strain (1:25 dilution; *Varilrix*™, GSK Vaccines) before overnight incubation with brefeldin A. Cells were stained for surface markers (CD3, CD4, and CD8), fixed, permeabilized, and stained with antibodies to each of the activation markers. Cells were then washed and analyzed by flow cytometry.

2.5. Assessment of humoral immune responses

Serum anti-VZV antibody concentrations were measured by enzyme-linked immunosorbent assay (ELISA) with the *Enzygnost*™ anti-VZV/IgG kit (Siemens Healthcare). The assay cut-off was 50 milli-international units (mIU)/mL. Serum anti-gE antibody concentrations were measured using a GSK in-house ELISA (Henogen). The assay cut-off was 109 ELISA units (EU)/mL.

2.6. Statistical analysis

Safety was analyzed on the total vaccinated cohort, which included all subjects who received at least one vaccine dose. Immunogenicity up to month 3 was analyzed on the according-to-protocol immunogenicity cohort, which included all subjects who received two vaccine doses according to protocol. The long-term persistence of immunogenicity was analyzed in all subjects enrolled at months 12–36.